HIGH RESOLUTION OF MILK PROTEINS OBTAINED BY GEL ELECTROPHORESIS

Zone electrophoresis on acrylamide gel, as described by Raymond (9), is particularly useful in determining the purity of protein preparations for genetic typing and the identification of protein fractions. It gives better resolution than paper electrophoresis, which has been used for genetic typing of β-lactoglobulin (2, 3, 4), α-lactalbumin (5, 6), and β-casein

(1), and starch gel, which has been used for genetic typing of α_s-casein (11).

Electrophoresis in acrylamide gel has a number of advantages over electrophoresis in starch gel: (a) Acrylamide gels are much easier to prepare than starch gels since they can be used 20 min after mixing and do not require heating, standing for 24 hr, and slicing; (b) acrylamide gel does not contain free carboxyl groups as does starch gel which may prevent the migration of some proteins; (c) higher voltages can be used for the cell used for electrophoresis in acrylamide than can be used in the flat cell described by Smithies; however, Smithies (10) has mentioned a water-jacketed cell in which gradients of 20 v per centimeter were used, giving better resolution of serum proteins than lower voltages.

EXPERIMENTAL PROCEDURE

A vertical gel cell with water-cooling was made following the design described by Raymond (9), which is commercially available. The buffer described by Raymond (9) for hemoglobin work proved to be the best of several tried. A stock buffer solution was made up from 2,700 ml of distilled water, 270 g of tris(hydroxymethyl) aminomethane, 35.1 g of disodium ethylenediaminetetraacetic acid, and 20.7 g of boric acid. This is diluted one to nine in making the gels and for filling the buffer vessels.

For the electrophoresis of casein all of the gels are made to contain 4.5 M urea for good resolution. Larger amounts of urea up to 7 M urea do not improve resolution. The acrylamide content must be at least 7%, since α_s-casein is not resolved in 5% gels. The gel is made by mixing 55 ml of stock buffer, 300 ml of water, 35 g of Cyanogum-41 gelling agent (Fisher Scientific Company[1]), 135 g of urea, and diluting to 500 ml. This solution is stable for several weeks in the refrigerator. The catalyst is ammonium persulfate and β-dimethylaminopropionitrile (DMAPN). Only the amine (0.5 ml) is added to the stock gel solution, and before use 0.3 g of ammonium persulfate is added to each 125 ml of solution, which is sufficient to fill the mold. The casting is done with the mold horizontal and the slot formers are placed in the open end. When the gel has set, the excess gel is removed from the open end, the cell placed on end, and the buffer vessels filled with the same buffer as in the gel, less urea.

Casein is dissolved in a one to three dilution of the stock buffer, with urea added to make it 7 M in respect to urea. This higher density compared to the buffer covering the upper end of the gel makes it possible for the sample to flow to the bottom of the slot without mixing, and gives a sharp line. One milligram of casein is dissolved in 0.1 ml of buffer and 0.05 ml of methyl red indicator (1%) is added. The dye visibly shows the layering in the slot and also marks the boundary of the fastest-moving casein component.

When the electrophoresis is started at pH 9.0 the positive electrode is attached to the lower buffer vessel and a voltage of 100 v is applied for the first 15 min until the casein has migrated into the gel. The voltage is then increased gradually up to 250 v while the current is held at 75 ma. The current then decreases through the remainder of the run. When the dye band reaches the bottom of the gel, the gel is removed and then stained. The dye is a saturated system of amidoschwartz in the Smithies solvent system (50 parts water, 50 methanol, and ten acetic acid) and dyeing lasts only 1 min. After washing in three changes of solvent overnight, the gel background is clear.

The first figure shows the dyed pattern of all the six phenotypes for β-caseins listed by Aschaffenburg (1). The whole caseins were run and only the slower-moving β-caseins were photographed. The lines appear somewhat broader than one would expect, when compared to the α_s zones.

Figure 2 shows all six phenotypes for α_s-casein. For these samples and the β-casein set we are indebted to Dr. Marvin Thompson, of this Laboratory, who has made an extensive

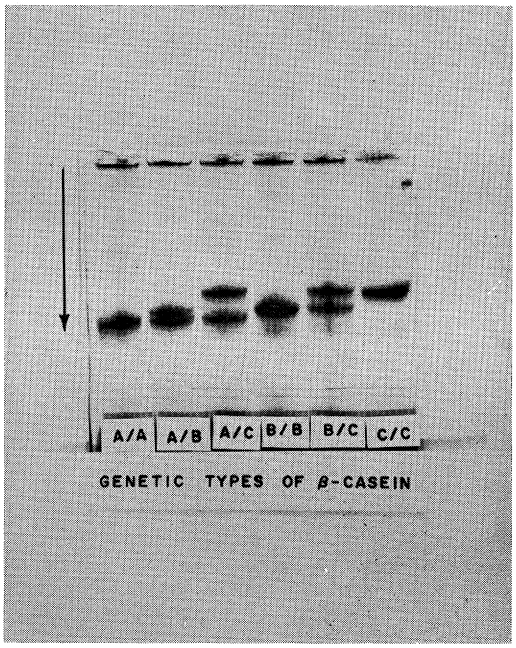

FIG. 1. Gel electrophoresis of β-casein variants using 7% acrylamide and 4.5 M urea at pH 9.0; α_s-caseins masked from negative; time, 5 hr at 250 v.

[1] It is not implied the USDA recommends the above company or its product to the exclusion of others in the same business.

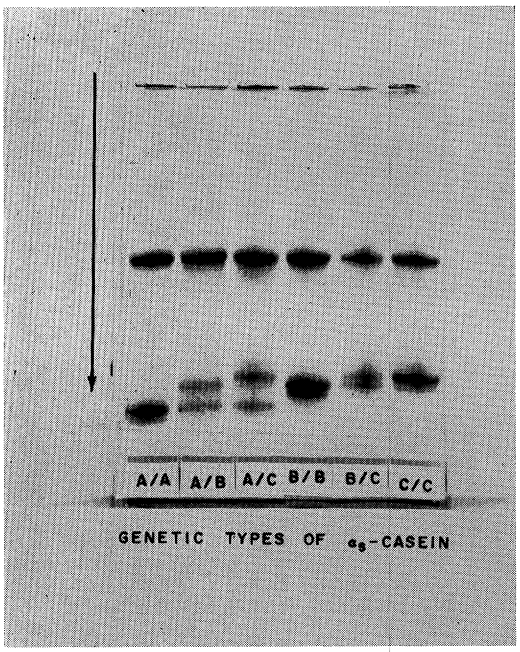

FIG. 2. Gel electrophoresis of α_s-casein variants using 7% acrylamide and 4.5 M urea at pH 9.0; all β-caseins are the same—A/A type; time, 5 hr at 250 v.

survey of casein types using starch gel electrophoresis (11). The α_s bands are narrow and well-defined. The typing of α_s-casein is aided by noting the number of closely following protein bands. When one of these is covered by the α_s band, the α_s is a C/C type. The original gels show this, but the photographs do not.

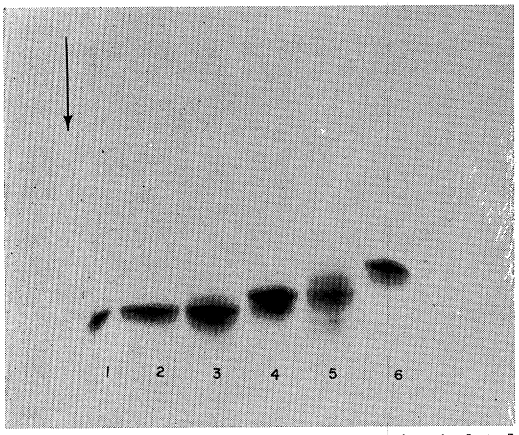

FIG. 3. Gel electrophoresis of β-caseins isolated by urea fractionation using 7% acrylamide and 4.5 M urea; time, 16 hr at 190 v. 1) Pooled β-casein, 2) A/A β-casein separated by urea fractionation, 3) A/A β-casein, 4) A/A β-casein, 5) B/B β-casein prepared by urea fractionation, 6) C β-casein separated from A/C by column chromatography.

Figure 3 shows the splitting in the β-casein band into two bands during long periods of electrophoresis. The β-caseins shown had been separated by urea fractionation and show traces of other proteins.

For typing of β-lactoglobulin and α-lactalbumin the urea-containing gels cannot be used, since these proteins show numerous components due to disulfide interchange. Furthermore, at 5% acrylamide concentration, these proteins migrate with the dye band and are not resolved. Good results were obtained with 8% gels and the boric acid–NaOH buffer used by Bell (4); these are shown in Figure 4. Here β-lactoglobulins A/A, B/B, and C/C are shown. The alpha lactalbumin in slot 6 was a twice recrystallized preparation which could be chromatographed to remove the other component. The α-lactalbumin migrates closely behind the β-lactoglobulin band. A concentrated acid milk whey is shown for comparison.

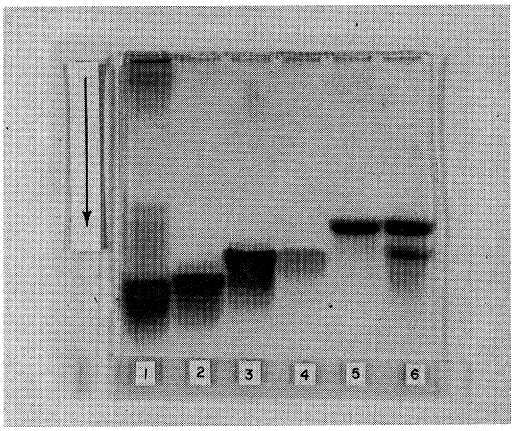

FIG. 4. Gel electrophoresis of whey proteins: 1) Whole whey, 2) A/A β-lactoglobulin, 3) B/B β-lactoglobulin, 4) C/C β-lactoglobulin, 5) α-lactalbumin prepared by chromatography, 6) α-lactalbumin twice recrystallized.

DISCUSSION

The amount of heat produced with high-voltage gradients is proportional to the product of voltage and amperage. When the tap water is very cold, much higher gradients can be used. We circulate ice water through the water jackets in summer. The effect of excessive wattage is to widen the bands and make the proteins at the center of the gel run faster. The distribution will be in a curve for identical samples.

Although Raymond (9) could not demonstrate any effect of pore size on the resolution of hemoglobins, the effect of the acrylamide concentration on the migration of milk proteins is marked. If the gels contain only 5% of acrylamide, α_s-casein runs in the dye band

and is not resolved. Further increase beyond 7 to 15% does not improve the resolution.

While the red proteins of Gordon and coworkers (7) and Groves (8) do not migrate on starch gel because of absorption effects, the red proteins do migrate on acrylamide gel and show a number of components.

We wish to thank Dr. R. Townend and M. L. Groves of this Laboratory for purified proteins. The C/C β-lactoglobulin was prepared by Dr. K. Bell (4).

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